BOOK REVIEWS

Evolution, 50(1), 1996, pp. 462–466

NON-NON-DARWINIAN EVOLUTION[1]

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Received October 9, 1995.

Evolutionary biology labors under the burden of an uncomfortably omniscient and prescient founder: Darwin was right about a great deal. He was wrong, however, about genetics, concocting his neo-Lamarkian theory of pangenesis at the same time as Mendel was conducting his experiments. Perhaps, therefore, population geneticists are the lucky ones in the evolutionary biology fold: at least, in Mendel, they know something that Darwin did not. The advent of electrophoresis in the mid-sixties and the discovery of extensive protein polymorphism in natural populations took them a step beyond merely knowing more than Darwin: they marginalized the old man, embracing what came to be dubbed "neutralism," a theory of molecular evolution that regards, in Kimura's words, polymorphism to be a "transient phase" of the stochastic extinction/fixation evolutionary process (see Kimura and Ohta 1971).

Neutral variants are mutations whose fitness effects on the individual are either zero or so small that drift overcomes the deterministic action of natural selection in governing their evolutionary fate. Under the neutral scheme, adaptive mutations (whether subject to balancing selection or positive selection for fixation) are vanishingly rare, and the vast majority of molecular differences among species results from the chance fixation of neutral variants. Nobody is claiming that all or even most mutations are neutral. It has been recognized for a long time, first in principle by Fisher (1930), and then from comparative analysis of protein and, later, nucleotide sequences of homologous genes from different species, that a large proportion of mutations are thoroughly deleterious and are accordingly weeded out by purifying selection. Thus, neutral mutations are the ones left over after the purifying selection filter—these are the mutations that can exist as polymorphisms with the potential to fix and contribute to divergence among species.

The Neutralist/Selectionist debate has framed most research in population genetics over the past 30 years. Both views have spawned extremes, pan-selectionists and pan-neutralists who see selection to be, respectively, omnipotent or irrelevant, but the debate has mainly been about a matter of degree: that is, what proportion of all mutations are adaptive? The two camps' positions are rather poorly defined here: neutralists consider very few mutations to be adaptive, selectionists rather more. Despite this fuzziness, the controversy has been fiercely contested and remains alive and well today. "Non-Neutral Evolution" seems to mark a turning point in the debate, a significant shift in how population geneticists perceive the Neutral Theory. I argue that, until recently, population geneticists tended to view the world neutrally: the genetic differences they saw among species and the polymorphisms they saw within species they tacitly assumed to be neutral. For this review, I take the term neutralism to characterize such a position. I am not referring to a simple scientific stance that a certain (high) proportion of all mutations are neutral, but rather to a more wide-ranging world view that deems adaptation trivial at the molecular level. "Non-Neutral Evolution" marks the return of adaptation to respectability in population genetics.

Kimura is justly regarded as the father and chief architect of the Neutral Theory, but the title of "Non-Neutral Evolution" refers, in fact, to an article published by King and Jukes (1969). It appeared in *Science*, and was perhaps as influential in establishing neutralism as part of the evolutionary mainstream as the more theoretical work of Kimura. The paper, titled "Non-Darwinian Evolution," started as follows, "Darwinism is so well established that it is difficult to think of evolution except in terms of selection for desirable characteristics and advantageous genes." Such a characterization of evolutionary opinion may seem self-serving, insofar as it creates a conveniently vulnerable straw man, but it nevertheless squares well with a biological world dominated by Dobzhansky and his acolytes. Dobzhansky believed polymorphism to be maintained by selection and saw, in general, the natural world through a lens of adaptation. His famous statement that "nothing in biology makes sense except in the light of evolution" (Dobzhansky 1973) was not made with the drift of neutral alleles in mind. It was this Dobzhansky versus Kimura/King-Jukes schism that became amplified into the Selectionist/Neutralist controversy.

King and Jukes pointed out that rates of nucleotide-based divergence exceed those of proteins, and argued that, because the redundancy of the genetic code results in a class of "synonymous" mutations which do not affect the amino acid sequence of a protein, truly neutral evolution at synonymous positions was responsible, at least in part, for this discrepancy. The genesis of the Neutral Theory thus had two empirical supports: that there was apparently too much protein (allozyme) variation in natural populations for selection to be solely responsible for its maintenance, and that there is a class of mutations that seem to be inaccessible to natural selection. The attractions of neutralism, however, went far beyond its empirical underpinning. Based on a simple set of diffusion equations, neutralism gave population geneticists something they had hitherto lacked, a model with measurable parameters that could be applied quantitatively to the distri-

[1] *Non-Neutral Evolution.* Brian Golding, ed. 1994. Chapman and Hall, London and New York. 249 pp. ISBN 0-412-05391-8. PB $39.50.

butions of gene frequencies in nature. Throwing selection out of the equations was immensely gratifying because selection coefficients are very difficult to estimate in the first place and may vary in a complex way across environments. Population genetics without selection had become a much more user-friendly discipline and, it seemed, a more rigorous one. The remaining parameters, particularly mutation rates and effective population sizes, were, in principle at least, not too hard to get a handle on.

There were, I think, other sociological reasons for the enthusiasm with which neutralism was embraced. With the technological advances that commenced with allozymes—the birth of wide-scale studies of molecular evolution—and are currently incarnated in a nice little earner for the manufacturers of automated DNA-sequencing machines, came biochemical systematics. Systematists have long sought characters that serve as true markers of genealogical history, characters that will not undergo selectively driven convergence. Biochemical markers, if neutral, fulfill these criteria, as well as having the great virtue of being available in very large quantities—even prokaryotes boast enough third positions to keep most systematists happy. The systematics community thus had a vested interest in the outcome of the neutralist/selectionist debate and implicitly (occasionally explicitly) treated biochemical characters as neutral.

Perhaps, given the joint desires of population geneticists for "hard" quantitative predictions and of systematists for reliable markers of history, it is not surprising that neutralism should enter evolutionary biology in such a way that, just a few years later, the first sentence of the King/Jukes paper would appear, in a population genetic context, positively atavistic. This process of assimilation was, however, facilitated by the shortcomings of the empirical tools available for distinguishing neutrality from selection. Neutralism provided a rigorous null hypothesis against which selective hypotheses could be tested and a great deal of effort was expended through the allozyme years in attempts to demonstrate selection by means of rejecting that null. It proved very difficult to reject.

There were, I believe, two basic reasons for this. First, protein electrophoresis is an inadequate and, at times, misleading tool for uncovering genetic variation. We now know, for example, that the two common alleles revealed by standard electrophoresis at the alcohol dehydrogenase locus in *Drosophila melanogaster* do indeed constitute the bulk of amino acid sequence polymorphism at the locus (Kreitman 1983) whereas a single electrophoretic "allele" at the *Drosophila pseudoobscura* esterase locus is, in fact, composed of several diverse proteins differing by as many as six amino acids (Veuille and King 1995). Electrophoresis thus, at best, adequately assayed protein variability and, at worst, massively underestimated it; a comparison of levels of polymorphism between the two above loci would produce artifactual results. Second, because the neutral process is stochastic, an expected outcome necessarily has a large variance, and it is accordingly hard to reject as a null hypothesis. The consistent failure to reject neutrality may have been due to either or both of these problems; alternatively, of course, both theory and data may have been sound and, in fact, the patterns under scrutiny were produced by genuinely neutral processes. Until the advent of more exhaustive techniques for dissecting genetic variation, in particular DNA sequencing, there was no way to determine which of these factors was responsible for a given result, but there seems nevertheless to have been a general consensus in favor of the last one, that evolution was indeed neutral. This was not, of course, a truly scientific decision: a failure to reject a null hypothesis should not result in the acceptance of that hypothesis. Neutralism had established an hegemony.

The ascendancy of neutralism in the molecular branches of evolutionary biology resulted in a bizarre schism between the two wings of the discipline, the organismic biologists and the people who preferred their organisms ground into a fine paste. Not only did the size of their NSF grants differ by some two orders of magnitude, but they also approached nature in surprisingly different ways. Organismic biologists continued to seek evidence of adaptation, while population geneticists concentrated instead on nonadaptive stochastic processes. The strangeness of this schism lies in its artificiality; the adaptive events being studied by the organismic biologists must inevitably have a molecular genetic basis—those events thus fall as much within the ambit of population genetics as within that of organismic biologists.

The situation was exacerbated by technological progress in molecular biology. What started as a separation between the two wings escalated to divorce. Because it is technically simple, the occasional protein electrophoresis gel was something that could be quite readily incorporated into a field biologist's research program. DNA, at least in the early days, was a full-time, specialist business and molecular evolutionists inevitably found themselves spending time with colleagues more interested in the mechanics of adenovirus gene expression than in the optimal diet of starlings. The departmental population geneticist was moved over to the brand new building with all those brushed stainless steel surfaces while their ecological colleagues remained stuck in that depression-era "temporary" building that had become permanent. Molecular biologists are interested in evolution because they know that the functionally significant parts of the molecules that they study are conserved. From the point of view of the molecular biologist interested in function, therefore, the parts of the gene which change—evolve, if you like—are junk: the emphasis is entirely on conservation. Such a typological emphasis served to reinforce the notion that what does change is neutral, unimportant from a selective point of view.

I think that, in many ways, because of the coincidence of events and sociological influences outlined above, neutralism derailed evolutionary biology. It provided population geneticists with a null hypothesis and offered many new and important insights into molecular and evolutionary processes but ultimately, in contributing to that schism within evolutionary biology, has yielded little more than a detour into unexciting territory. Evolutionary biology is about changes that matter, changes that contribute to the extraordinary fit of organisms with their environments. It is, I believe, the brief of evolutionary biologists to explain the immense and fantastic diversity we see in nature. What kept Darwin awake at night was the problem of how to cobble together an eyeball by natural selection. The flux and flow through evolution of

neutral "A"s, "G"s, "C"s, and "T"s in the intron of an eyeball-determining gene are inconsequential; what matters are the mutations that result in that triumph of optical design. Rather like the drift of those neutral "A"s, "G"s, "C"s, and "T"s, however, the ruling paradigm in evolutionary biology seems to wax and wane. We have seen from King and Jukes' first sentence that, courtesy of Dobzhansky, Darwinism reigned supreme at the time of neutralism's birth. "Non-Neutral Evolution," I think, marks, as Gettysburg did for the South, the turn of the tide against neutralism. It bears testimony to the latest phase of population genetics in which new statistical approaches and molecular data have combined to relegate neutralism to its original role of null hypothesis, one which can now be tested with some hope of rejection.

The book, which is edited by Brian Golding and includes his brief preface, is the proceedings of a workshop convened by the Canadian Institute for Advanced Research in 1993. It was published in 1994, which means that Golding is to be congratulated for coercing his authors into producing manuscripts while the topics are still current (this alone sets it apart from other symposium volumes). It consists of 19 papers on both theoretical and empirical aspects of population genetics and molecular evolution (the subtitle is "Theories and Molecular Data"). Considering that the whole lot is squeezed into fewer than 250 pages means that the papers are of sensible length, short enough to be worth reading in their entirety. Any choice of 19 papers (and 29 authors) to represent a field inevitably results in apparently arbitrary exclusions (and equally arbitrary inclusions) but, on balance, the book is respectably representative of what is going on in the field.

There are plenty of omissions that will cause offense: plants, for example, are not part of the new non-neutral world, whereas the whole place is thoroughly overrun with *Drosophila*, which feature centrally in nine of the papers. As a drosophilist, I am doubtless biased here, but the preponderance of *Drosophila* papers seems to be an accurate reflection of the areas in which interesting new work is being carried out. This, inevitably, is partly due to Dobzhansky's legacy but is also related to the efficiency with which *D. melanogaster* made the transition from model genetic organism to model molecular biological organism. Only one paper (Schaeffer) concentrates on Dobzhansky's species of choice, *D. pseudoobscura*, whereas several discuss the results of work on *D. melanogaster*. This is because population geneticists took advantage of the techniques and innovations developed by their colleagues interested in *D. melanogaster* for developmental or molecular genetic reasons. Thus, the organism of choice for evolutionary studies today—it even graces the cover of "Non-Neutral Evolution"—is a pragmatically acquired hand-me-down from other branches of biology.

Drosophila melanogaster has a few other advantages as a study organism for evolutionary biologists. It can be captured anywhere in the world with little more than a rotten banana and a net for equipment and it is typically present in numbers that make population-sampling the work of five minutes. Beyond these trivial virtues (and its very significiant virtues as a laboratory animal), *D. melanogaster* really is a pretty poor choice of organism for evolutionary study. A human commensal, its natural habitat through most of its range is the trash can. We know virtually nothing of its ecology in Africa, its purported center of origin, and have only the sketchiest feel for its population dynamics even in the best-studied North American populations. Furthermore, because it has apparently undergone a recent range expansion in response to human migrations, many of the populations that have been subject to evolutionary study, especially those in North America, are probably still in a state of post-introduction consolidation; they have not yet attained an evolutionary equilibrium. The application of equilibrium population genetic models (i.e., almost all population genetic models) to such populations is thus inappropriate. Surely, from an evolutionary point of view, we would be well advised to study a species whose ecology poses interesting biological questions, rather than questions about fruit truck schedules, and one which we may reasonably assume to be currently in a state of evolutionary stationarity, having occupied a given undisturbed range for a respectably long period of time? I hope that "Non-Neutral Evolution" also represents *D. melanogaster*'s Gettysburg in evolutionary studies; the technology which was previously largely tied to *melanogaster* is now readily transferable to other organisms, and the time has come to forsake the molecular biologists' hand-me-down.

What accounts for the dimming of the neutralist star? DNA sequencing has finally provided us with data that have the necessary resolution and statistical power to test properly (i.e., with some hope of rejection) the neutral null hypothesis. In addition, a number of statistical approaches have been introduced which facilitate testing. It is hardly worth extolling here the advantages of DNA sequence data over allozymes, but I will emphasize one point: unlike allozymes, DNA sequence permits accurate quantification of divergence among species as well as levels of polymorphism within species. Having both types of information at hand permits us to test the neutral expectation of a correspondence between levels of polymorphism within species and levels of divergence between species. The rationale is as follows: a gene, such as a histone, which is highly constrained by purifying selection, will diverge only very slowly between species and will also exhibit very little polymorphism, whereas an unconstrained gene, say a pseudogene, will diverge rapidly and exhibit high levels of polymorphism. The insight of Hudson, Kreitman and Aguadé (1987) was that, under neutrality, the ratio of histone divergence to histone polymorphism should equal the ratio of pseudogene divergence to pseudogene polymorphism and this expectation forms the basis of what has become known, in an unfortunate fit of acronym-enthusiasm, as the HKA test. Departures from the neutral expectation can be caused by a number of selective factors. For instance, in genomic regions of low recombination, a selective fixation can result, through hitchhiking, in the elimination of all polymorphism.

The HKA test and subsequent tests of neutrality, especially Tajima's test (1989), which is based on the expected frequency distribution under neutrality, have, as "Non-Neutral Evolution" bears witness, been widely applied. The sample of genes studied is biased towards the possibility of detecting departures from neutrality. The HKA test was originally applied to the alcohol dehydrogenase locus in *D. melanogaster* where allozyme studies had already revealed the presence of

a protein polymorphism subject to balancing selection. Several later studies targeted regions of the genome with reduced recombination rates in order to inflate the probability with which a selective event could be detected. Through hitchhiking, a selective event affects a large region in an area of low recombination, whereas hitchhiking is minimal when recombination is high and the effect of a selective event is very local (see the chapter by Aguadé and Langley). Because these studies were designed to maximize the chances of finding evidence of selection, we cannot, at this stage, make any quantitative statement about how much of the genome is evolving selectively; such an estimate will have to await studies of randomly chosen regions. However, one conclusion is clear: that the null hypothesis of neutrality is not impregnable. There are now many instances of departures from neutrality, and selection, we suppose, is responsible for those departures.

We now have a number of cases in which we can infer the action of the kind of selection that is deemed by neutralism to be vanishingly rare, either balancing or positive directional selection. Population geneticists are once again back into the adaptation fold with their organismic colleagues. Where to now?

The empirical studies featured in "Non-Neutral Evolution" follow what has been called the "static" approach to population genetics. This entails inferring the action of selection (or lack of it) from the distribution of genotypes in nature. The book ignores the alternative "functional" approach, which explores the significance, in terms of individual fitness, of allelic differences. Functional studies thrived in the allozyme era when, by means of enzymological assays, investigators tried to measure fitness differences among alleles. There are, however, two problems which dog all functional analyses. First, the functional assay (e.g., the rate of catalysis of an enzyme on a certain substrate) may lack the sensitivity to detect differences which, though small, are nevertheless selectively significant. Second, we have to re-create natural conditions in these experiments, which is very difficult. We may thus find differences that may in fact be irrelevant in nature because our assay conditions are never encountered in the organism's natural environment: how, in short, do biochemical differences among alleles translate into individual fitness differences?

Given these problems, it is perhaps not surprising that functional studies are no longer widely pursued. Nevertheless, the detection of selection by static means is not where the investigation should stop; rather, that should serve as a stepping off point for functional study. With today's methods of detailed physiological/genetic analysis, it may be possible to overcome some of the problems inherent to such a project. Cathy Laurie's work on the *D. melanogaster* alcohol dehydrogense polymorphism serves as an excellent example (e.g., Laurie and Stam 1994). The time has come to investigate in detail the mechanistic basis of the selection whose genetic footprint we can detect by static analysis.

The current enthusiasm for the static approach has not only severely diminished the role of functional analysis in evolutionary genetics but has also produced a rather formulaic approach to the problem of detecting selection. The formula is as follows: pick your locus and sequence it from multiple lines of *D. melanogaster*, sequence it from a single *D. simulans* line, and apply the HKA (using the alcohol dehydrogenase 5′ region for comparison), Tajima (1989), and Fu and Li (1993) tests. One problem with this is the blind acceptance of the significance levels given for each test. What is needed (and seems to be slowly forthcoming) is a substantial set of simulations to investigate the robustness of these tests with respect to their assumptions: does a statistically significant HKA result imply the action of selection or is it an artifact brought about by the violation of one of the equilibrium assumptions underpinning the test? A second problem associated with following such a formula is that, in doing so, we miss many opportunities for profitable evolutionary analysis. Frustrations stemming from the weakness of allozyme data forced that era's static evolutionists to invoke ingenious ancillary arguments such as geographical ones; hence, for example, the interest in allozyme clines, whether latitudinal or more local. In embracing the HKA formula, we seem to have lost sight of these innovations and their power. The priority seems to lie with the technological aspects of the study, the quality of the sequence being paramount, while careful sampling of study organisms no longer merits serious attention. Two papers in "Non-Neutral Evolution," however, demonstrate that geographical arguments remain alive and well.

Stephan summarizes his work on patterns of polymorphism in Asian populations of *D. ananassae*, in which he found evidence of selectively driven divergence between two geographically isolated populations in a region of the genome that is subject to little recombination. The suggestion, therefore, is that the two populations have undergone separate recent selective events, implying possible adaptation to local conditions by each population.

McDonald points out, as have others before him, that contrasting patterns of geographic differentiation among loci or among classes of polymorphism can be used to infer the action of selection, or lack of it. The power of DNA sequence analysis lies in the different classes of mutations available for comparison at a single locus. We may thus infer the action of selection when, say, we find a cline in an amino acid polymorphism superimposed on a homogeneous distribution of synonymous polymorphisms: selection is maintaining the cline in the face of gene flow strong enough to homogenize the distribution of neutral markers.

The paper by Aquadro, Begun and Kindahl provides the best illustration of the power and scope of contemporary molecular population genetics. The paper starts with the startling observation, made by Begun and Aquadro (1992), that levels of polymorphism in the *Drosophila* genome are correlated with recombination rates: heterozygosity is high in regions of frequent recombination and low where recombination is rare. This pattern they originally attributed to hitchhiking in response to positive selection for adaptive mutations (i.e., selective sweeps), hitchhiking being more extreme in the regions of low recombination. However, Charlesworth, Morgan and Charlesworth (1993) proposed an alternative explanation for the observed pattern. Their suggestion, also based on hitchhiking, was that purifying selection against deleterious mutations caused the reduction in polymorphism in regions of reduced recombination. They argued that this

constant elimination of deleterious mutations resulted in what may be viewed as a reduction in the effective population size of that particular region of the genome and that the extent of that reduction is a function of its rate of recombination (i.e., the extent to which it is subject to hitchhiking) as well as the net rate of deleterious mutation (see the paper by Hudson and Kaplan). Thus, put simply, a region's effective population size is correlated with its recombination rate. It is, furthermore, a classic Neutral Theory result that levels of polymorphism are related to effective population size. Thus we have an alternative explanation for the Begun/Aquadro observation: regions of low recombination are subject to extensive hitchhiking in response to purifying selection against deleterious mutations and accordingly have a small effective population size, which we know to be characterized by low levels of polymorphism.

Aquadro, Begun and Kindahl confront the background selection alternative. They point out that the two competing explanations for their recombination rate/heterozygosity correlation, background selection and selective sweeps, make different predictions about levels of variation in regions of equivalent recombination on the X and autosomal chromosomes. Because genes on the X are hemizygous in males, purifying selection is more effective against recessive deleterious mutations on the X than on autosomes. The mechanics of background selection are such that its impact is reduced if deleterious mutations are not permitted to drift up in frequency (as recessives will do at autosomal loci). Thus we expect to see higher levels of variation under background selection on the X relative to autosomes at loci of equivalent recombination rates. X-linked hemizygosity also has an impact under selective sweeps insofar as recessive advantageous mutations are fixed more rapidly than their autosomal counterparts, which means that, under selective sweeps, we expect to see a reduction of variation at loci with equivalent recombination rates on the X relative to autosomes. The two models thus make qualitatively different predictions for the X/autosome comparison: loci of equivalent recombination rates on the X should have more polymorphism than their autosomal counterparts under background selection whereas, under selective sweeps, they should have less. Aquadro, Begun and Kindahl present preliminary data for a comparison of levels of polymorphism between *D. melanogaster* X and third chromosome loci of equivalent recombination rates. They find X-linked loci to be less variable than their autosomal counterparts, supporting the selective sweep explanation of their polymorphism level/recombination rate correlation.

That I find the Aquadro, Begun and Kindahl article to be the highlight of this collection is probably largely attributable to my being a *Drosophila* population geneticist. Although, as previously noted, "Non-Neutral Evolution" has a definite *Drosophila* bias, it is not as monochromatic as perhaps this review has made it sound: oysters (Beckenbach) and halophilic archaebacteria (Dennis) make appearances, and even vertebrates get a look-in in the final, largely theoretical, chapter by Takahata. If nothing else, it serves as a summary of the way in which the static brand of evolutionary genetics is being practiced in *Drosophila*; my hope is that these papers may serve to inspire people to take the approaches developed in flies and to apply them to their own infinitely more charismatic (and ecologically interesting) organisms. The book is affordable, even if you don't have a grant to charge book purchases to, and it has the unusual virtue of carrying very little in the way of we-have-to-produce-something-for-this-volume papers, though DeSalle and Vogler present a paper that seems to be little more than a rehash of a paper by Davis and Nixon (1992). There are, in addition, a number of minor editorial transgressions, products presumably of the rush to press. Particularly irritating are the lack of abstracts for some chapters plus the failure to implement a consistent bibliographic policy, with some sets of references being listed alphabetically and others in the order of citation.

Time will tell whether this volume will really serve as a marker for the latest shift in the shifting balance of evolutionary paradigms. Whatever history's conclusion, this collection illustrates well what I regard to be a healthy treatment of neutralism. It provides us with a marvelous null hypothesis which, with the arrival of large scale DNA data, is finally testable. In addition, neutral mutations will continue to serve us well as indicators of history, whether of individuals within populations, populations within species, or species within higher taxa. The paradigm shift I see is a subtle one—it does not, for example, involve a wholesale refusal to recognize that any mutations evolve in a neutral manner. Rather, it is a shift of emphasis: molecular evolution is not uniformly neutral (or, in King and Jukes' language, non-Darwinian) but, like morphological evolution, frequently adaptive, non-neutral, non-non-Darwinian.

Literature Cited

Begun, D. J., and C. F. Aquadro. 1992. Levels of naturally occurring DNA polymorphism correlate with recombination rates in *D. melanogaster*. Nature 356:519–520.

Charlesworth, B., M. T. Morgan, and D. Charlesworth. 1993. The effect of deleterious mutations on neutral molecular variation. Genetics 134:1289–1303.

Davis, J. I., and K. C. Nixon. 1992. Populations, genetic variation, and the delimitation of phylogenetic species. Syst. Biol. 41:421–435.

Dobzhansky, Th. 1973. Nothing in biology makes sense except in the light of evolution. Am. Biol. Teach. 35:125–129.

Fisher, R. A. 1930. The genetical theory of natural selection. Oxford University Press, Oxford.

Fu, Y.-X., and W.-H. Li. 1993. Statistical tests of neutrality of mutations. Genetics 133:693–709.

Hudson, R. R., M. Kreitman, and M. Aguadé. 1987. A test of neutral evolution based on nucleotide data. Genetics 116:153–159.

Kimura, M., and T. Ohta. 1971. Protein polymorphism as a phase of molecular evolution. Nature 229:467–469.

King, J. L., and T. H. Jukes. 1969. Non-Darwinian evolution. Science 164:788–798.

Kreitman, M. E. 1983. Nucleotide polymorphism at the alcohol dehydrogenase locus of *Drosophila melanogaster*. Nature 304: 412–417.

Laurie, C. C., and L. F. Stam. 1994. The effect of an intronic polymorphism on alcohol dehydrogenase expression in *Drosophila melanogaster*. Genetics 138:379–385.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Veuille, M., and L. M. King. 1995. Molecular basis of polymorphism at the esterase-5B locus in *Drosophila pseudoobscura*. Genetics 141:255–262.

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